THE FERTILIZING POWER OF SPERM DILUTIONS OF ARBACIA

By Frank R. Lillie

DEPARTMENT OF ZOOLOGY, UNIVERSITY OF CHICAGO

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My previous studies of fertilization led to the conclusion that initiation of development depends upon activation of a substance produced by the egg and located in its cortex. This substance, for which I propounded the name *fertilizin*, possesses two side-chains involved in fertilization, one of which unites with receptors borne by the spermatozoon, the other with the receptors borne by the egg. The latter is the fertilization reaction proper; it was postulated that any agent that may activate the fertilizin, as do the sperm receptors, so as to cause its ovophile side-chain to combine with the egg-receptors may act as a parthenogenetic agent. Fertilization and parthenogenesis are thus brought under one point of view with reference to initiation of development.

Certain data concerning the fertilizin of Arbacia, such as time of origin, its location in the egg, its disappearance after fertilization, etc., are known owing to its property of agglutinating spermatozoa of the same species, which serves as indicator. Some of its chemical and physical properties have also been studied to a certain extent. But up to the present the very existence of the so-called sperm receptors has remained hypothetical. On any theory of fertilization it is necessary to postulate the existence of a spermatic substance that induces development of the egg. But in spite of a considerable number of investigations, the existence of such a substance still remains an hypothesis. The present contribution presents demonstrative evidence, though of a negative sort, concerning this substance.

The phenomena, on which I rely for my conclusion, concern the fertilizing power of sperm suspensions of graded dilutions. On the basis of the usual supposition that a single active spermatozoon may fertilize an egg of its own species, all of the eggs should be fertilized in a series of sperm suspensions of increasing dilutions up to the place in the series in which each egg receives only a single spermatozoon. Beyond this the percentage of eggs fertilized should fall off at a certain rate to a vanishing point.

An approximate realization of this may be obtained if the interval between preparation of the more dilute sperm suspensions and their use in fertilization be made as short as possible. This may be done by the addition of eggs to measured quantities of sea-water, followed by the

addition of a sufficient quantity of an accurately determined sperm suspension stirred in quickly to reach the desired dilution. The final dilution is then made in the presence of eggs, and the age of the final dilution is therefore practically zero with reference to the fertilization reaction.

Under these conditions 100% of the eggs may fertilize up to a dilution of about 1/3000 of a 1% sperm suspension. The curve of fertilizing power of the sperm suspensions measured in terms of the percentage of eggs fertilized then falls off slowly to 1/24,000 of 1% sperm, then rapidly to about 1/300,000%, then slowly again to about 1/90,000,000% where, however, about 1% of fertilization may still take place. Accompanying observations showed that beyond a dilution of about 1/5000 of a 1% sperm suspension only a single spermatozoon can possibly be concerned in the fertilization of each egg.

One obtains exceedingly contrasting results if a series of sperm dilutions in powers of 2 is made, beginning with 1%, by first transferring a certain amount of the 1% suspension to a second crystal of the series and adding an equal amount of sea-water, proceeding similarly from crystal 2 to crystal 3, and so on down the series. In such a case one finds that fertilization runs out absolutely from about a 1/64 dilution of 1% sperm suspension to 1/1024% in different cases. No comment is needed to emphasize the contrast.

As many as 20 to 40 active spermatozoa are found in association with each egg at 1/128%, which may be, however, absolutely ineffective. So that in an experiment running out in the seventh crystal of the series it would appear that a greater number of spermatozoa than this is required to fertilize an egg.

These results suggest at first glance that the order of adding eggs and sperm to the sea-water may be of significance. This is, however, not the case. The repeated handling of the sperm in successive half dilutions is also not the main cause for the result. Thus it would appear that the only real difference between the fertilizing power of the sperm in these cases is a time factor. In the first case the final dilution is made in the presence of the eggs; in the second case 20 to 30 minutes is consumed in the preparation of the sperm dilutions before the eggs are added.

The time factor is the real explanation as will be shown immediately. But at first sight this did not seem a very reasonable explanation for the following reasons: In the first place the time involved has never been considered sufficient to reduce fertilizing power of sperm; and in the

second place the original 1% sperm suspension was shown in several experiments to be capable of fertilizing a high percentage of eggs at 1/30,000 dilution or less, after a longer interval of time. If the sperm suspensions lose their fertilizing power with time, it must be that the significance of time in this respect varies inversely to concentration.

This conclusion was abundantly verified by the following tests: A quantity of sperm suspension of a given concentration is prepared and divided in several equal amounts in a series of crystals; the same quantity of eggs is then added at time intervals to the crystals of the series, and the percentages of fertilization estimated by careful counts. Fourteen different grades of dilution between 1/300 and 1/240,000 of 1% sperm were thus measured. Loss of fertilizing power was shown in all of the suspensions thus tested in less than one hour; and in general the rate of loss increased with each successive dilution. The actual data are to be published elsewhere. Here we may summarize the results in the following table, showing the approximate time required A, for 66% loss of fertilizing power, B, for complete loss, at six different dilutions.

Dilution of sperm	1/1200%	1/3000%	1/6000%	1/30000%	1/60000%	1/120000%
A. 66% loss	32 min.	7 min.	5 min.	3 min.	2-3 min.	1 min.
B. 100% loss	64 min.	24 min.	?	20 min.	20? min.	7 min.

Suspensions of higher concentrations, than those included in the table exhibited a much slower rate of loss, which was measured by another method, showing that from 1/4% down, loss occurs in increasing amount within a period of 100 minutes.

Other possible factors than time influencing the fertilizing power of sperm suspensions are on the whole relatively slight in this series of experiments. There is a certain natural variation in different lots of ova and sperm, which is no doubt responsible for some irregularities in the data. Another factor is that of egg-concentration; but a series of determinations showed that the actual variations due to this cause, occurring in the experiments themselves, are not of significance.

There are two modes of explanation of these results theoretically possible, viz: First, that the loss of fertilizing power is due to loss of motility of the spermatozoa; in the first place this theory does not agree with the observation, that the spermatozoa lose their fertilizing power before they lose their motility; in the second place it renders the increase of rate of loss with dilution incomprehensible because on *a priori* grounds the exact opposite would be expected; in the third place as a result of several lines of work the theory that the fertilization reaction is primarily a function of motility of the spermatozoon has been given up; penetra-

tion of the ovum by the spermatozoon is due to the inception of the fertilization reaction, and not the reverse, as was previously assumed. We cannot therefore accept this theory.

The second theory is that the spermatozoa lose their activating substance, sperm receptors in my terminology, which agrees very well with the demonstrated ineffectiveness in spite of the observed persistent motility. That the rate of loss should increase with dilution is to be expected if we regard the loss of the sperm receptors as a diffusion phenomenon; and, if we regard it as an active process of secretion, we should expect such a result owing to general increase of functional activity under conditions which approach more nearly the normal.

The second mode of explanation, which I have actually adopted, fits in with the necessary postulate that the spermatozoon bears such a substance, and with the fact that the spermatozoon carries out the initial fertilization reaction while it is still intact and external to the ovum. The postulated activating substance of the spermatozoon must be liberated before penetration, and these experiments give us some idea as to the manner of its liberation.

Glaser (*Biol. Bull.* **26,** 84–91; 1914) has recently maintained that more than one spermatozoon is necessary for fertilization of the same form which I have studied. His observations may also find their explanation under the same point of view, inasmuch as he was not aware of the significance of the time factor in inseminations with highly dilute sperm.

We thus obtain the following additional point of view with reference to fertilization: the spermatozoon arriving at the egg while still intact liberates an activating substance which initiates the fertilization reaction; as one consequence among others of this reaction the spermatozoon is taken up by the egg, and completes the process of fertilization in its interior. This point of view is consistent, as far as it goes, with my own theory of the fertilization reaction; and it is also perfectly consistent with Loeb's quite different point of view.

My previous experiments had shown that eggs lose a certain substance in sea-water (fertilizin) which is necessary for their fertilization; fertilized eggs no longer produce this substance and are incapable of fertilization. Both eggs and spermatozoa, therefore, contain substances, more or less liable to loss, which are necessary for fertilization. The mechanism of fertilization cannot possibly, therefore, be regarded in the simple manner postulated by Loeb's theory. The existence of parthenogenesis demonstrates the efficacy under given conditions of the egg-substance alone; we must therefore regard the spermatic substance essentially as an activator of the fertilizin of the egg.

There remains of course the problem of identifying the free activator in the medium of the sperm suspension by its only known mode of operation, that of fertilizing the ovum. This problem, over which several investigators have broken their weapons, appears in a somewhat different light as a result of these experiments; and new experiments should therefore be undertaken.

VARIATION IN BACTERIA

By Edwin O. Jordan

DEPARTMENT OF HYGIENE AND BACTERIOLOGY, UNIVERSITY OF CHICAGO

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The bearing of slight physiological differences upon the classification of bacteria and upon the phenomena of infection has made the occurrence of variation among bacteria fully as conspicuous as in the higher forms of life. During the past ten years many observations have been recorded upon the extent and nature of bacterial variability. In these studies some confusion has arisen through the difficulty of distinguishing true variations from the development of latent characteristics, and from environmental modification.

By the term 'latent characteristics' is meant those qualities or properties that are dormant in the organism or cell and are manifested only in response to definite external influences. Thus, certain bacteria form spores in the presence, but not in the absence, of oxygen; some bacteria are known that develop conspicuous capsules when growing in the animal body, but lack these envelopes in part or altogether in artificial media; according to Wright, animal fluids seem to be essential for the production of the characteristic clubs of Actinomyces colonies. The sudden appearance in this way of a definite morphological character cannot be looked upon as an instance of variation. Such a manifestation is merely an immediate response to changed conditions of life and is of exactly the same kind as the marked difference in the aquatic and terrestrial forms of Polygonum amphibium referred to by DeVries, or as shown in the transformation of the shrimp Artemia salina into what some writers consider another species, Artemia milhausenii, when the former is transferred to water of a greater degree of saltiness. In no sense is the awakening of such a dormant character to be confounded with true variation. In other words the power to produce certain structures or certain physiological effects exists ready formed in the specific